REMARKS

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. Also submitted herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a disk copy of the Substitute Sequence Listing. The disk copy of the Sequence Listing, file "0147-0199P.ST25", is identical to the paper copy, except that it lacks formatting.

The amendments to the Specification are being made to identify the sequences by their SEQ ID NOS. No new matter is introduced by these amendments.

Extension of Time

Pursuant to 37 C.F.R §§ 1.17 and 1.136(a), the applicant respectfully petitions for a two (2) month(s) extension of time for filing a response in connection with the present application and the requirement fee of $195.00 is enclosed herewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

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LRS/CAV
0147-0199P

Attachments:
Disk Copy of Sequence Listing
Paper Copy of Sequence Listing
Copy of Notice to Comply
Version with Markings to Show Changes Made

(Rev. 03/27/01)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please replace the paragraph beginning on page 17, line 19 with the following rewritten paragraph:

--Figure 1.2: DNA sequence (SEQ ID NO: 49) designated CTI that was cloned into the multiple cloning site of the Bluescript KS vector (GenBank® accession number X52327) by using the restriction sites XbaI and SalI in order to increase the number of possible cloning sites. CTI-derived restriction enzyme cleavage sites are shown.--

Please replace the paragraph beginning on page 19, line 18 with the following rewritten paragraph:

--Figure 1.8: DNA-sequence (SEQ ID NOS: 50-51) of the double-stranded oligonucleotide designated ACCGS15BAM with single-stranded overhangs compatible with those of restriction enzymes BspEI and BamHI. Amino acids (SEQ ID NO: 52) encoded by the nucleotide sequence are shown.--

Please replace the paragraph beginning on page 21, line 4, with the following rewritten paragraph:

--Figure 3.1: DNA- and protein sequence (SEQ ID NOS: 53-54) of the human D4.5. heavy chain variable region (V_H of the human anti-17-1A-antibody VD4.5VK8). Number indicate the nucleotide (nt) positions, amino acids are presented in the single letter code. CDR1 includes nt 91 to nt 105, CDR2 nt 148 to nt 198, CDR3 nt 292 to nt 351.--

Please replace the paragraph beginning on page 21, line 9, with the following rewritten paragraph:

--Figure 3.2: DNA- and protein sequence (SEQ ID NOS: 55-56) of the human kappa 8 light chain variable region (V_L of the human anti-17-1A-antibody VD4.5VK8). Numbers indicate the nucleotide (nt) positions, amino acids are presented in single letter code. CDR1 includes nt 70 to nt 102, CDR2 nt 148 to nt 168, CDR3 nt 265 to nt 294.--

Please replace the paragraph beginning on page 21, line 31, with the following rewritten paragraph:

--Figure 3.4: NS3 Frame: DNA-sequence (SEQ ID NO: 57) designated L-F-NS3Frame that was cloned into the multicloning site of the vector Bluescript-KS-CTI (Figure1.2) by using the restriction sites EcoRI and SalI in order to increase the number of possible cloning sites. Cloning sites derived from L-F-NS3Frame are shown.--

Please replace the paragraph beginning on page 22, line 20, with the following rewritten paragraph:

--Figure 5.2: DNA sequence (SEQ ID NO: 58) of the multiple cloning site of pComb3H5BHis showing important restriction enzyme cleavage sites as well as the amino acid sequence (SEQ ID NO: 59) of the Glycine-Serine-linker and that of the N2-domain of the gene III-product of filamentous phage.

The DNA-sequence encoding the N2-domain starts at nt 19 and ends at nt 411.--

Please replace the paragraph beginning on page 23, line 22, with the following rewritten paragraph:

--Figure 6.3: DNA- and protein-sequence (SEQ ID NOS: 60-61) of the mouse scFv fragment 3-1. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 360 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 406 and ends at nt 726.--

Please replace the paragraph beginning on page 23, line 29, with the following rewritten paragraph:

--Figure 6.4: DNA- and protein-sequence (SEQ ID NOS: 62-63) of the mouse scFv fragment 3-5. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 372 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 418 and ends at nt 753.--

Please replace the paragraph beginning on page 24, line 4, with the following rewritten paragraph:

--Figure 6.5: DNA- and protein-sequence (SEQ ID NOS: 64-65) of the mouse scFv fragment 3-8. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 360 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 406 and ends at nt 726.--

Please replace the paragraph beginning on page 24, line 11, with the following rewritten paragraph:

--Figure 6.6: DNA- and protein-sequence (SEQ ID NOS: 66-67) of the mouse scFv fragment 4-1. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 360 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 406 and ends at nt 744.--

Please replace the paragraph beginning on page 24, line 18, with the following rewritten paragraph:

--Figure 6.7: DNA- and protein-sequence (SEQ ID NOS: 68-69) of the mouse scFv fragment 4-4. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 360 followed by

a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 406 and ends at nt 726.--

Please replace the paragraph beginning on page 24, line 25, with the following rewritten paragraph:

--Figure 6.8: DNA- and protein-sequence (SEQ ID NOS: 70-71) of the mouse scFv fragment 4-7. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 372 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 417and ends at nt 753.--

Please replace the paragraph beginning on page 25, line 1, with the following rewritten paragraph:

--Figure 6.9: DNA- and protein-sequence (SEQ ID NOS: 72-73) of the mouse scFv fragment 5-3. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 348 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 394 and ends at nt 717.--

Please replace the paragraph beginning on page 25, line 8, with the following rewritten paragraph:

--Figure 6.10: DNA- and protein-sequence (SEQ ID NOS: 74-75) of the mouse scFv fragment 5-10. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 360 followed by a $(G_4S_1)_3$-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 406 and ends at nt 744.--

Please replace the paragraph beginning on page 25, line 15, with the following rewritten paragraph:

--Figure 7: DNA- and protein-sequence (SEQ ID NOS: 76-77) of the mouse scFv fragment 5-13. Numbers indicate the nucleotide (nt) positions, amino acids (aa) are presented in single letter code. The first four aa of the V_H-fragment are encoded by the plasmid pComb3H5BHis. The encoding DNA sequence for the V-region of the heavy chain starts at nt 1 and ends at nt 360 followed by a (G4S1)3-linker. The coding DNA sequence for the V-region of the kappa chain starts at nt 406 and ends at nt 744.--

Please replace the paragraph beginning on page 29, line 5, with the following rewritten paragraph:

--A protein was constructed that consists of the single-chain Fv fragment (scFv) of the murine anti 17-1A antibody M79 and the extracellular part of the human costimulatory protein CD80 (B7-1) connected by a $(Gly_4Ser_1)_1$ linker (Figure 1.1). The M79 antibody was obtained as described by Göttlinger (1986) Int.J.Cancer:38, 47-53. The M79 scFv fragment was cloned as

described by Mack. Proc. Natl. Acad. Sci. U.S.A.. 92 (1995) 7021-7025. The complete plasmid was cloned in several steps. First a poly-linker designated CTI was inserted into the Bluescript KS vector (GenBank® accession number X52327) using the restriction enzyme cleavage sites XbaI and SalI (Boehringer Mannheim). The introduction of the polylinker CTI provided additional cleavage sites as well as the sequence encoding the $(Gly_4Ser_1)_1$, linker a six-amino acid histidine tag and a stop codon as shown in Figure 1.2..The vector Bluescript KS + CTI was prepared by cleavage with the restriction enzymes EcoRV and XmaI (Boehringer Mannheim and New England Biolabs) in order to ligate it (T4 DNA, Ligase Boehringer Mannheim) with the M79 scFv fragment cleaved by EcoRV and BspEI (New England Biolabs). The resulting vector Bluescript KS+CTI+M79 scFv again was cleaved with EcoRI (Boehringer Mannheim) and BspEI in order to insert the CD80 DNA-fragment which was previously prepared using the same enzymes. Prior to subcloning, the CD80 fragment was obtained by polymerase chain reaction (PCR) using specific oligonucleotide primers complementary to the 5´ and 3´ ends of the nucleotide sequence encoding the extracellular part of CD80 (Freeman G.J et.al. J.Immunol.143,(1989) 2714 - 2722.). These primers also introduced an EcoRI and a BspEI cleavage site (5´CD80 Primer (SEQ ID NO: 1): 5´GCA GAA TTC ACC ATG GGC CAC ACA CGG AGG CAG 3´; 3´CD80 Primer (SEQ ID NO: 2): 5´TGG TCC GGA GTT ATC AGG AAA ATG CTC TTG CTT G 3´) The cDNA template used for this PCR was prepared by reverse transcription of the total RNA prepared from the Burkitt-lymphoma cell line Raji according to standard procedures (Sambrook, Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press, cold Spring Habour, New York (1989)).--

Please replace the following paragraph on page 32, line 14, with the following replacement paragraph:

--To change the arrangement of the Ig variable regions within the M79scFv fragment from V_L/V_H to V_H/V_L a two step fusion PCR using oligonucleotide primers 5´V_HB5RRV (SEQ ID NO: 3):AGG TGT ACA CTC CGA TAT C(A,C)A (A,G)CT GCA G(G,C)A GTC (A;T)GG, 3´V_HGS15, 5´V_LGS15 ,3´V_LBspE1 (for sequences of the three last oligonucleotides see Example 2.1) was performed according to the procedure described by Mack, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7021-7025 (see also Example 2.1.) The PCR-fragment encoding the V_H/V_L-scFv-fragment was cleaved with the restriction enzymes EcoRV/BspEI and inserted into the vector Bluescript KS + CTI already prepared by cleavage with EcoRV/XmaI (see Example 1.1.). Next, the inverted M79scFv (V_H/V_L) fragment was excised with the restriction enzymes BspEI/SalI and introduced into the plasmid pEF-DHFR+CTI + CD80-M79scFv (V_L/V_H) using BspEI/SalI thus replacing the M79scFv- V_L/V_H fragment (see Figure 1.3.2.). Transfection and cell culture procedures were carried out as described above. Analysis of antigen binding was performed using the described 17-1A-ELISA (Example 1.1.2.). However, no 17-1A binding activity of the alternatively arranged CD80-M79scFv-construct could be detected. Results are shown in Figure 1.7.--

Please replace the paragraph beginning on page 34, line 5, with the following rewritten paragraph:

--A protein was constructed that consists of the single-chain Fv fragment (scFv) of the anti 17-1A antibody M74 and the

costimulatory protein CD80 connected by a (Gly_4Ser_1) linker (Figure1.1). The M74 antibody was obtained as described by Göttlinger (1986) Int. J. Cancer: 38, 47-53. V_L and V_H of M74 were cloned from the total RNA of the corresponding hybridoma cell line as described by Orlandi (1989) Proc. Natl. Acad. Sci. USA 86, 3833-3837 and sequenced. The plasmids containing V_L and V_H of the M74 antibody respectively were used as templates for a two-step fusions-PCR resulting in M74 scFv-fragments with either the domain arrangement V_L/V_H or the alternative arrangement V_H/V_L. Regarding the V_L/V_H arrangement, the primers for M74 V_L were 5´V_LB5RRV (SEQ ID NO:4) (5´AGG TGT ACA CTC CGA TAT CCA GCT GAC CCA GTC TCC A3´) and 3´V_LGS15 (SEQ ID NO:5) (5´GGA GCC GCC GCC GCC AGA ACC ACC ACC ACC TTT GAT CTC GAG CTT GGT CCC3´), for M74 V_H 5´M74V_HGS15 (SEQ ID NO: 6) (5´GGC GGC GGC GGC TCC GGT GGT GGT GGT TCT CAG GT(GC) (AC)A(AG) CTG CAG (GC)AG TC(AT) GGA CCT GAG CTG GTG AAG CCT GGG GCT TCA GTG AAG ATT TCC TGC 3´) and 3´V_HBspEI (SEQ ID NO: 7) (5´AAT CCG GAG GAG ACG GTG ACC GTG GTC CCT TGG CCC CAG3`). Regarding the $V_H/V_L$-arrangement the primers for M74 $V_H$ were 5´M74$V_H$EcoRV (SEQ ID NO: 8) (5´TCC GAT ATC (AC)A(AG) CTG CAG (GC)AG TC(AT) GGA CCT GAG CTG GTG AAG CCT GGG GCT TCA GTG AAG ATT TCC TGC 3´) and 3`V_HGS15 (SEQ ID NO: 9) (5´GGA GCC GCC GCC GCC AGA ACC ACC ACC ACC TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CCA G 3´), for M74 VL 5´V_LGS15 (SEQ ID NO: 10) (5´GGC GGC GGC GGC TCC GGT GGT GGT GGT TCT GAC ATT CAG CTG ACC CAG TCT CCA3´) and 3´VLBspEI (SEQ ID NO: 11) (5´AAT CCG GAT TTG ATC TCG AGC TTG GTC CC3´). In the first PCR step the corresponding V_H-and-V_L fragments were obtained using the following PCR-program: denaturation at 94 °C for 5 min., annealing at 37°C for 2 min., elongation at 72°C for 1 min. for the first cycle; denaturation at 94°C for 1 min., annealing at 37°C for 2 min., elongation at

72°C for 1 min. for 6 cycles; denaturation at 94°C for 1 min., annealing at 55°C for 1 min., elongation at 72°C for 45 sec and 18 cycles ; terminal extension at 72°C for 2 min.).--

Please replace the paragraph beginning on page 36, line 13, with the following rewritten paragraph:

--In a further Example a human anti-17-1A antibody (VD4.5VK8) selected in vitro by the phage display method from a combinatorial antibody library was chosen to analyse its antigen-binding activity at the C-Terminus of a bifunctional single-chain construct as in Examples 1, 2 and 4 and as illustrated in Figure1.1. The V_H -and V_L-chain of VD4.5VK8 were available in the form of cloned DNA fragments with known nucleotide sequence (Figure 3.1 and 3.2) and served as template molecules for PCR using the following primers: for V_H: 5´V_H1357 (SEQ ID NO: 12) 5´-AGG TGC AGC TGC TCG AGT CTG G-3, and 3´huV$_H$BstEII (SEQ ID NO: 13) 5´-CTG AGG AGA CGG TGA CC´-3; for VL: 5´VK3 (SEQ ID NO: 14) GAG CCG CAC GAG CCC GAG CTC GTG (AT)TG AC(AG) CAG TCT CC-3´, and 3´huVkBsiWI/SpeI (SEQ ID NO: 15) 5´-GAA GAC ACT AGT TGC AGC CAC CGT ACG TTT (AG)AT-3´). The V_H- respectively V_L-chains were introduced into a newly constructed vector designated pComb3H5BHis and described in Example 5. VD4.5VK8 VH was subcloned with XhoI and Bst EII, VD4.5VK8 V_L with SacI and SpeI resulting in the plasmid: pCOMB3H5BHis+VD4.5VK8 V_H+V_L. By using the pComb3H5BHis-vector a fusion PCR was no longer necessary to obtain a scFv-antibody fragment with the domain arrangement V_H/V_L.--

Please replace the paragraph beginning on page 39, line 9, with the following rewritten paragraph:

--The pComb3H vector was cleaved with NheI and a double stranded oligonucleotide with suitable ends was inserted by ligation. The double stranded oligomer encoding the six His residues was created through annealing of the two 5'-phosphorylated primers His6s and His6as (at 94°C, 10 min.; 65 °C, 30 min.; 52 °C 30 min. and 30 °C 10 min.).

His6s (SEQ ID NO: 16): 5'-CTAGCCATCACCATCACCATCACA-3'

His6as (SEQ ID NO: 17): 5'-CTAGTGTGATGGTGATGGTGATGG-3'--

Please replace the paragraph beginning on page 40, line 4, with the following rewritten paragraph:

--Primer sequences:

5Bfors (SEQ ID NO: 18):
5'-GCAGCTGGTCGACAAATCCGGAGGTGGTGGATCCGAGGTGCAGCTGC-3'

5BForas(SEQ ID NO: 19):
5'-TCGAGCAGCTGCACCTCGGATCCACCACCTCCGGATTTGTCGACCAGCTGCAGCT-3'--

Please replace the paragraph beginning on page 40, line 21, with the following rewritten paragraph:

--Primer sequences:

5Bbacks (SEQ ID NO: 20):

5′-
TCGAGCCCGGTCACCGTCTCCTCAGGTGGTGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTG
GTTCTGAGCTCGGGA-3′

5Bbackas (SEQ ID NO: 21):

5′-
CTAGTCCCGAGCTCAGAACCACCACCACCGGAGCCGCCGCCGCCAGAACCACCACCACCTGAGG
AGACGGTGACCGGGC-3′--

Please replace the paragraph beginning on page 41, line 23, with the following rewritten paragraph:

--Primer sequences:

5′ N2 SalI (SEQ ID NO: 22)-:—5′-GGTGTCGACACTAAACCTCCTGAGTACGG-3′

3′N2 BspEI (SEQ ID NO: 23)-:—5′-GCCTCCGGAAGCATTGACAGGAGGTTGAGG-3′--

Please replace the paragraph beginning on page 46, line 19, with the following rewritten paragraph:

--Positive clones were detected by PCR-based colony screening with the following primers:

5' B7-1 (SEQ ID NO: 24) 5'- GCA GAA TTC ACC ATG GGC CAC ACA CGG AGG CAG-3'

3' mu VK (SEQ ID NO: 25) 5'-TGG TGC ACT AGT CGT ACG TTT GAT CTC AAG CTT GGT CCC-3'--

Please replace the paragraph beginning on page 50, line 7, with the following rewritten paragraph:

--The CD54 antigen known as ICAM-1 (Intercellular adhesion molecule-1) belongs to the Ig-superfamily. It is a heavily glycosilated protein which is expressed on many lymphoid cells, e.g. dendritic-cells. A more detailed description was published by Simmons D. et.al. Nature 331 (1987) 624-626. The cDNA template was obtained by reverse transcription of the total RNA prepared from TPA-stimulated HL-60-cells. To amplify the extracellular region of CD54, specific primers for the 5´and 3´end were used. These primers also introduced the restriction cleavage-sites EcoR1 and BspE1 (5´ ICAM (SEQ ID NO: 26): CTC GAA TTC ACT ATG GCT CCC AGC AGC CCC CG and 3´ICAM (SEQ ID NO: 27): GAT TCC GGA CTC ATA CCG GGG GGA GAG CAC).--

Please replace the paragraph beginning on page 51, line 16, with the following rewritten paragraph:

--CD58 also known as LFA-3 (Lymphocyte Function-Associated Antigen) is a protein belonging to the Ig-superfamily and is the counterreceptor of CD2. A more detailed description was published by Wallner B.P. et.al. J.Exp.Med 166 (1987) 923-932). The cDNA template was obtained by reverse transcription of the total RNA prepared from U937 cells. To amplify the extracellular region of CD58 and to introduce the restriction enzyme cleavage sites Xba1 and BspE1, specific 5´and 3´primers were used (5`LFA-

3 (SEQ ID NO: 28) AA TCT AGA ACC ATG GTT GCT GGG AGC GAC G and 3´LFA-3 (SEQ ID NO: 29)-AAG TCC GGA TCT GTG TCT TGA ATG ACC GCT GC). The further cloning and expression procedure was the same as described above for the CD54 constructs except that XbaI instead of EcoRI was used due to an internal EcoRI-site within the CD58-DNA-fragment and a dam-methylase deficient E.coli-strain was used in order to prevent blocking of the BspEI site at the 3´-end of the CD58-fragment due to an overlapping dam-site. The finally resulting transfected CHO cells (pEF-DHFR-CTI-CD58- anti 17-1A 4-7, pEF-DHFR-CTI-CD58- anti 17-1A 5-3 and pEF-DHFR-CTI-CD58- anti 17-1A 5-10) were grown for selection in nucleoside free α-MEN medium supplemented with 10% dialyzed FCS and 2mM L-glutamine. The expression of these constructs was subsequently increased by gene amplification induced by the addition of the DHFR-inhibitor methotrexate (MTX) to a final concentration of 20nM as described (Kaufman, Methods Enzymol. 185 (1990), 537-566).--

Please replace the paragraph beginning on page 52, line 12, with the following rewritten paragraph:

--The CD86 costimulatory protein also known as B7-2 belongs to the Ig superfamily. It is a heavily glycosylated protein of 306 amino acids. A more detailed description was published by Freeman G.J.et.al. Science 262 (1993) 909-911. The cDNA template was obtained by reverse transcription of the total RNA prepared from the Burkitt-Lymphoma cell line Raji. To amplify the extracellular region of CD86 specific 5´and 3´primers (5`B7-2 (SEQ ID NO: 30): 5´AAG TCT AGA AAA TGG ATC CCC AGT GCA CTA TG 3´, 3`B7-2 (SEQ ID NO: 31): 5´AAT TCC GGA TGG GGG AGG CTG AGG

GTC CTC AAG C ´3) were used. These primers also introduce Xba1 and BspE1 cleavage sites which were used to clone the CD86 PCR-fragment into the vector Bluescript KS-CTI-M79scFv (VL/VH) (see Example 1). The further cloning and expression procedure was the same as described above for the CD54-construct except that XbaI instead of EcoRI was used due to an internal EcoRI-site within the CD86-DNA-fragment. The finally resulting transfected CHO cells (pEF-DHFR-CTI-CD86- anti 17-1A 4-7, pEF-DHFR-CTI-CD86-anti 17-1A 5-3 and pEF-DHFR-CTI-CD86- anti 17-1A 5-10) were grown for selection in nucleoside free α-MEN medium supplemented with 10% dialyzed FCS and 2mM L-glutamine. The expression of these constructs was subsequently increased by gene amplification induced by the addition of the DHFR-inhibitor methotrexate (MTX) to a final concentration of 20nM as described (Kaufman, Methods Enzymol. 185 (1990), 537-566).--

Please replace Table 1 on page 54, with the following rewritten Table:

--Table 1: Primer sets for the amplification of the VH- and VK-DNA-fragments (5' to 3')

murine V heavy chain:

5' primer

(SEQ ID NO: 32)	MVH1	5'-(GC)AGGTGCAGCTCGAGGAGTCAGGACCT-3'
(SEQ ID NO: 33)	MVH2	5'-GAGGTCCAGCTCGAGCAGTCTGGACCT-3'
(SEQ ID NO: 34)	MVH3	5'-CAGGTCCAACTCGAGCAGCCTGGGGCT-3'
(SEQ ID NO: 35)	MVH4	5'-GAGGTTCAGCTCGAGCAGTCTGGGGCA-3'
(SEQ ID NO: 36)	MVH5	5'-GA(AG)GTGAAGCTCGAGGAGTCTGGAGGA-3'
(SEQ ID NO: 37)	MVH6	5'-GAGGTGAAGCTTCTCGAGTCTGGAGGT-3'

(SEQ ID NO: 38) MVH7 5'-GAAGTGAAGCTCGAGGAGTCTGGGGGA-3'

(SEQ ID NO: 39) MVH8 5'-GAGGTTCAGCTCGAGCAGTCTGGAGCT-3'

3' primer (SEQ ID NO: 40)

MUVHBstEII 5'-TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG-3'

murine V kappa chain:

5' primer

(SEQ ID NO: 41) MUVK1 5'-CCAGTTCCGAGCTCGTTGTGACTCAGGAATCT-3'

(SEQ ID NO: 42) MUVK2 5'-CCAGTTCCGAGCTCGTGTTGACGCAGCCGCCC-3'

(SEQ ID NO: 43) MUVK3 5'-CCAGTTCCGAGCTCGTGCTCACCCAGTCTCCA-3'

(SEQ ID NO: 44) MUVK4 5'-CCAGTTCCGAGCTCCAGATGACCCAGTCTCCA-3'

(SEQ ID NO: 45) MUVK5 5'-CCAGATGTGAGCTCGTGATGACCCAGACTCCA-3'

(SEQ ID NO: 46) MUVK6 5'-CCAGATGTGAGCTCGTCATGACCCAGTCTCCA-3'

(SEQ ID NO: 47) MUVK7 5'-CCAGTTCCGAGCTCGTGATGACACAGTCTCCA-3'

3' primer (SEQ ID NO: 48)

MUVKHindIII/BsiWI 5'-TGGTGCACTAGTCGTACGTTTGATCTCAAGCTTGGTCCC-3'

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